Amendments to the Specification

Please amend the paragraph bridging lines 1-3 on page 2 in the following manner:

The present ~~inventor~~ **invention** has identified a new (sub)type of avian astrovirus isolatable from chickens ~~(CastV-2)~~ **(CAstV-2)** that is immunologically distinct from the known chicken astrovirus ANV ~~(CastV-1)~~ **(CAstV-1)** and from other avian astroviruses.

Please amend the paragraph bridging lines 4-8 on page 2 in the following manner:

Therefore, the present invention provides a chicken astrovirus type 2 (CAstV-2), characterised in that the virus is the CAstV deposited under accession no. I-2932 at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institute Pasteur, Paris, France or an immunological related **[[CastV]]** **CAstV** that is able to induce antiserum that ~~neutralises~~ **neutralizes** the deposited virus.

Please amend the paragraph bridging lines 9-17 on page 2 in the following manner:

As mentioned-above, the existing avian astroviruses are immunologically distinct and the ~~CastV-2~~ **CAstV-2** as defined above has also been found to be immunologically distinguishable from previously known avian (astro)viruses. The new ~~CastV-2~~ **CAstV-2** can be used for the preparation of poultry vaccines and diagnostic tests. A ~~CastV-2~~ **CAstV-2** according to the invention can be obtained from the Depository Institute (CNCM of the Institute Pasteur) or can be isolated from infected animals in the field and can be identified as such by reaction with specific antisera raised against the deposited virus in an immunological assay as described in the Examples. A virus ~~neutralisation~~ **neutralization** assay is particularly suitable for the identification of a ~~CastV-2~~ **CAstV-2** according to the present invention.

Please amend the paragraph bridging lines 18-27 on page 2 in the following manner:

It is generally accepted that biological variation exists in nature between organisms of the same type. For the purpose of this invention the deposited **[[CastV]]** **CAstV** is considered to be the reference strain of ~~**CastV-2**~~ **CAstV-2** with which the other ~~**CastV-2**~~ **CAstV-2** strains are immunologically related. The virus ~~**neutralisation**~~ **neutralization** assay and immunofluorescence (IF) assay are widely used in the art for determining the presence or absence of an immunological relationship between (avian) viruses. Typical virus ~~**neutralisation**~~ **neutralization**- and IF assays are described in the Examples and are also disclosed in Mockett et al. (Avian Pathology 22, 751-770, 1993), McNulty et al. (Avian Pathology 19, 75-87, 1990) and Nersessian et al. (Am. J. Vet. Res. 50, 1475-1480, 1989). In Tables 1 and 2 it is shown that ~~**CastV-2**~~ **CAstV-2** strains form a homogeneous serogroup of chicken astroviruses that is immunological distinct from the known avian astroviruses.

Please amend the paragraph bridging lines 28-32 on page 2 in the following manner:

Therefore, a **[[CastV]]** **CAstV** is considered to belong to the present invention in case the avian astrovirus is immunological related to the deposited **[[CastV]]** **CAstV**, that is to say in case it is able to induce antiserum that is capable of ~~**neutralising**~~ **neutralizing** the deposited virus in a virus ~~**neutralisation**~~ **neutralization** assay. A ~~**neutralisation**~~ **neutralization** assay that can be used to determine the immunological relationship is described in Example 2 below.

Obviously, the antiserum to be used in the virus ~~**neutralisation**~~ **neutralization** assay should be of appropriate quality. Methods for the preparation of such antiserum are described in Example 2.

Please amend the paragraph bridging lines 1- 8 on page 3 in the following manner:

Generally, appropriate antiserum raised against a live **[[CastV]]** **CAstV** can be prepared by inoculating 3 to 4 weeks old SPF chickens orally with a live virus strain having an infectious titre between $10^{2.0}$ - $10^{9.0}$ pfu/animal; more preferably between $10^{3.0}$ - $10^{6.0}$ pfu/animal. Blood can be

collected 3 to 4 weeks after infection, preferably 4 weeks after infection. Chickens may also be re-infected with the same live virus strain 3 to 4 weeks after the first infection with approximately the same dose as used in the first infection. Blood is collected between 2 and 4 weeks after the second infection.

Appropriate antiserum raised against inactivated **[[CastV]]** **CAstV** can be obtained by inoculating 3 to 4 weeks old SPF chickens subcutaneously or intramuscularly with the inactivated virus preparation and an adjuvant. The infectious titre of the preparation before inactivation may be between $10^{7.0}$ - $10^{11.0}$ pfu/animal; more preferably between $10^{8.0}$ - $10^{10.0}$ pfu/animal. Blood can be collected 3 to 4 weeks after inoculation, preferably 4 weeks after inoculation. Chickens may also be re-inoculated with the inactivated virus preparation 3 to 4 weeks after a first inoculation with the live- or inactivated virus preparation. Blood is collected between 2 and 4 weeks after the second inoculation.

An antiserum of appropriate quality typically comprises a ~~**neutralising**~~ **neutralizing** antibody titre of ≥ 256 against the homologous virus.

Preferably, the present invention provides a chicken astrovirus type 2 (CAstV-2), characterised in that the virus is the CAstV deposited under accession no. I-2932 at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institute Pasteur, Paris, France or an immunological related **[[CastV]]** **CAstV** that has a percent relatedness (%R) with the deposited virus of at least 32, more preferable at least 50, most preferably at least 70 as determined by virus cross-neutralisation and calculated according to the method of Archetti and Horsefall (J. Exp. Med. 92, 441-462, 1950). "R-values" of 32-50 between a test virus and the deposited **[[CastV]]** **CAstV** indicate a clear immunological relationship between the two, whereas a "R-value" of 50-70 and at least 70 indicate minor or little/no immunological difference, respectively.

Further ~~**characterisation**~~ **characterization** of the CAstV-2:

Treatment with ether and IDUR: The virus is a stable agent resistant to ether and its growth was not inhibited by IDUR – indicating it is a RNA virus.

Please amend the paragraph bridging lines 1-7 on page 5 in the following manner:

As demonstrated in the Examples, the ~~CastV-2~~ CAstV-2 according to the invention displays an immunogenic make-up that is not observed before. Therefore, the new ~~CastV-2~~ CAstV-2 may form the basis of a new type of avian astrovirus vaccine that can effectively protect poultry against disease conditions resulting from the infection by the new ~~CastV-2~~ CAstV-2. Hence, another aspect of this invention is a vaccine for use in the protection of poultry against disease caused by avian astrovirus infection, characterised in that the vaccine comprises a ~~CastV-2~~ CAstV-2 as defined above, together with a pharmaceutical acceptable carrier or diluent.

Please amend the paragraph bridging lines 8-9 on page 5 in the following manner:

The ~~CastV-2~~ CAstV-2 according to the invention can be incorporated into the vaccine as a live attenuated or inactivated virus.

Please amend the paragraph bridging lines 15-18 on page 5 in the following manner:

Briefly, a susceptible substrate is inoculated with a ~~CastV-2~~ CAstV-2 according to the invention and propagated until the virus replicated to a desired infectious titre after which ~~CastV-2~~ CAstV-2 containing material is harvested, optionally inactivated, and mixed with a pharmaceutical acceptable carrier or diluent.

Please amend the paragraph bridging lines 19-27 on page 5 in the following manner:

Every substrate which is able to support the replication of ~~CastV-2~~ CAstV-2 can be used to prepare a vaccine according to the present invention, including primary (avian) cell cultures, such as chicken embryo fibroblast cells (CEF) or chicken embryo liver cells (CEL). Usually, cells are

incubated at 39°C and can be infected after 24-48 hours at which time the medium is removed from the cultures and the virus inoculated onto the culture and allowed to absorb for 20-40 minutes. Fresh medium is used to refeed the culture, which is then incubated for a further 24-96 hours. At this time the infected cell culture fluids and the infected cells may be harvested separately or together. In order to liberate cell-associated virus the infected cells are sonicated for 3x5 seconds. If desired cell debris may be removed by filtration or centrifugation.

Please amend the paragraph bridging lines 28-29 on page 5 in the following manner:

Therefore, in a further embodiment the present invention provides a cell culture infected with a ~~CastV-2~~ CAstV-2 as defined above.

Please amend the paragraph containing line 30 on page 5 in the following manner:

The new ~~CastV-2~~ CAstV-2 can also be propagated in embryonated chicken eggs.

Please amend the paragraph bridging lines 31-35 on page 5 in the following manner:

Attenuation of the ~~CastV-2~~ CAstV-2 can be obtained by standard serial passaging of the virus in cell cultures, for example in primary cell cultures (e.g. CEL cell culture) or established cell lines that support the replication of the virus. In this case, passage levels between 5-150, preferably between 20-50, may be used. Obviously, also naturally occurring attenuated strains of the new ~~CastV-2~~ CAstV-2 may be used for the preparation of a live vaccine.

Please amend the paragraph bridging lines 13-17 on page 6 in the following manner:

Although administration by injection, e.g. intramuscularly, subcutaneously or **in ovo** of the live vaccine according to the present invention is possible, the vaccine is preferably administered by an inexpensive mass application route commonly used for poultry vaccination. For ~~CastV-2~~ CAstV-2 vaccination this route includes drinking water, spray and aerosol vaccination.

Please amend the paragraph bridging lines 18-19 on page 6 in the following manner:

Alternatively, the present invention provides a vaccine comprising the new ~~CastV-2~~ CAstV-2 in an inactivated (killed) form.

Please amend the paragraph bridging lines 23-24 on page 6 in the following manner:

A vaccine containing the inactivated ~~CastV-2~~ CAstV-2 can, for example, comprise one or more of the above-mentioned pharmaceutically acceptable carriers or diluents suited for this purpose.

Please amend the paragraph bridging lines 30-34 on page 6 in the following manner:

The vaccine according to the invention comprises an effective dosage of the ~~CastV-2~~ CAstV-2 as the active component, i.e. an amount of immunising ~~CastV-2~~ CAstV-2 material that will induce immunity in the vaccinated birds against challenge by a virulent virus. Immunity is defined herein as the induction of a statistically significant higher level of protection in a population of birds after vaccination compared to an unvaccinated group.

Please amend the paragraph bridging lines 5-7 on page 7 in the following manner:

Although, the ~~CastV-2~~ CAstV-2 vaccine according to the present invention may be used effectively in chickens, also other poultry such as turkeys may be successfully vaccinated with the vaccine. Chickens include broilers, pullets, reproduction stock and laying stock.

Please amend the paragraph bridging lines 8-13 on page 7 in the following manner:

It is possible to induce an antibody response by vaccinating parent stock (e.g. at 12-16 weeks of age for broiler breeder stock) with or without prior vaccination with the ~~CastV-2~~ CAstV-2 vaccine as young stock. The antibodies induced by such a vaccination regime are high and are passed through to the progeny in the yolk to provide protection against early challenge with field

astrovirus. It is also possible to vaccinate young birds, in particular with a live attenuated vaccine, early in life, preferably at day-old, to induce active immunity.

Please amend the paragraph bridging lines 14-15 on page 7 in the following manner:

The invention also includes combination vaccines comprising, in addition to the ~~CastV-2~~ CAstV-2 described above, one or more vaccine components of other pathogens infectious to poultry.

Please amend the paragraph bridging lines 19-23 on page 7 in the following manner:

A ~~CastV-2~~ CAstV-2 according to the invention can also be used for the preparation of diagnostic assays. The ~~CastV-2~~ CAstV-2 can be used as an antigen in immuno assays for the detection of ~~CastV-2~~ CAstV-2 antibodies in samples of animals suspected of being infected by that virus. Alternatively, the ~~CastV-2~~ CAstV-2 can be used to raised antisera to be used in a diagnostic assay, i.e. for the detection of ~~CastV-2~~ CAstV-2 antigen.

Please amend the paragraph bridging lines 3-9 on page 8 in the following manner:

EXAMPLES

Example 1 Isolation of CAstV-2 strains

~~CastV-2~~ CAstV-2 – VDU/As1

Tracheal and vent swabs taken from 10 broiler chicks (5 days old) with ~~diarrhoea~~ diarrhea were washed with PBS and the total volume (0.2ml) of fluid from each sample was inoculated on to CEL cultures. After 6 days an astrovirus induced cytopathic effect was observed in the cultures inoculated with one of the tracheal swab samples. Reovirus and adenovirus was isolated from the other samples. This astrovirus was plaque-purified three times, a single plaque being taken from a plate with less than 10 plaques on it.

Please amend the paragraph bridging lines 10-15 on page 8 in the following manner:

~~CastV-2~~ CAstV-2 – VDU/As2

Ten, 1ml heparinised blood samples were taken from one-week-old broiler chicks that showed uneven growth. The white blood cells were separated from the samples after centrifugation (using a bench centrifuge) and approximately 0.1ml of packed white cells were inoculated into the medium of each of two 48 hours old chick embryo liver (CEL) 6cm cell culture tissue plates.

Please amend the paragraph bridging lines 21-29 on page 8 in the following manner:

~~CastV-2~~ CAstV-2 – VDU/As3

The virus was isolated from a pool of small intestines ~~(homogenised)~~ (homogenized) (SI) from three 3-week-old chicks with ~~diarrhoea~~ diarrhea. The SI samples were filtered using a coarse filter and diluted 100-fold with PBS containing 5 x normal cell culture levels of penicillin and streptomycin. The samples were centrifuged (bench centrifuge) to remove particulate debris and then the supernatant was diluted in 10-fold steps to 10^{-6} and each dilution inoculated onto four CEL plates. After absorption for 40 minutes plates were overlaid with medium containing 0.9% agar and two refeed with normal fluid maintenance medium.

Four days later typical reovirus plaques and CPE were observed in dilutions up to 10^{-3}.

Please amend the paragraph at line 15 on page 9 in the following manner:

Example 2 Immunological ~~characterisation~~ **characterization** of ~~CastV-2~~ **CAstV-2**

Please amend the paragraph bridging lines 18-23 on page 9 in the following manner:

10 x 2-week-old SPF chicks (White Leghorn) were inoculated with 10^6 pfu/chick of live ~~CastV-2~~ **CAstV-2** orally. After a further 4 weeks each chick was inoculated subcutaneously with 10^9 pfu of ~~CastV-2~~ **CAstV-2** mixed with incomplete Freudts adjuvant (Vol 0.5ml/chick). Blood was collected 4 weeks after the last inoculation and the serum separated and stored at –20°C. The serum ~~neutralising~~ **neutralizing** titres varied between 256 and 1024. Sera against other avian astroviruses were prepared according to similar protocols.

Please amend the paragraph bridging lines 2-6 on page 10 in the following manner:

The plaque reduction test was used for ~~CastV-2~~ **CAstV-2**, ANV (except for the ANV experiment described in Table 1b), TastV and DVH-2 antibody test. Antisera were diluted with phosphate buffered saline in two-fold steps. An equal volume of each antiserum dilution (usually 0.5 ml) was mixed with an equal volume of a virus preparation diluted to contain approximately 200 plaque forming units 0.1ml.

Please amend the paragraph bridging lines 2-3 on page 12 in the following manner:

Results

Table 1a Immunological relationship between avian astroviruses ~~CastV-2~~ **CAstV-2**, ANV and TastV

Please amend the table bridging lines 4-15 on page 12 in the following manner:

			VIRUS		
			~~CastV-2~~ CAstV-2 VDU/AS2)	AN V	TastV (strain TEV)
ANTISERUM	~~CastV-2~~ CAstV-2 (VDU/AS2)	~~Neutralisation~~ **Neutralization**	256*	<16	ND
		Immunofluorescence	128	<8	ND
		Gel Diffusion	+	-	ND
	ANV	~~Neutralisation~~ **Neutralization**	<16	256	ND
		Immunofluorescence	<16	32	ND
		Gel Diffusion	-	+	ND
	TastV (strain TEV)	~~Neutralisation~~ **Neutralization**	<16	ND	ND
		Immunofluorescence	<16	ND	ND

Please amend the table heading bridging lines 1-2 on page 13 in the following manner:

Table 1b Immunological relationship between avian astroviruses ~~CastV-2~~ CAstV-2, ANV and DVH-2

Please amend the table bridging lines 4-15 on page 12 in the following manner:

VIRUS

			~~CastV-2~~ **CAstV-2** (VDU/AS1)	ANV	DVH-2
ANTISERUM	~~CastV-2~~ **CAstV-2** (VDU/AS1)	~~Neutralisation~~ **Neutralization**	1600*	<10	<10
		Immunofluorescence	256	<10	<10
		Gel Diffusion	+	-	-
	ANV	~~Neutralisation~~ **Neutralization**	<10	2048	<10
		Immunofluorescence	<10	64	<10
		Gel Diffusion	-	+	-
	DVH-2	~~Neutralisation~~ **Neutralization**	<10	<10	1600
		Immunofluorescence	<10	<10	128
		Gel Diffusion	-	-	+

Key * = serum titre
\- = Negative reaction
\+ = Positive reaction
ND = Not done

Please amend the table heading at line 1 on page 14 in the following manner:

Table 2 Immunological relationship between ~~CastV-2~~ CAstV-2 strains

			VIRUS		
			VDU/AS1	VDU/AS2	VDU/AS3
ANTISERA	VDU/AS1	~~Neutralisation~~ Neutralization	256*	128	256
		Immunofluorescence	64	64	64
		Gel Diffusion	+	+	+
	VDU/AS2	~~Neutralisation~~ Neutralization	128	128	256
		Immunofluorescence	32	32	32
		Gel Diffusion	+	+	+
	VDU/AS3	~~Neutralisation~~ Neutralization	256	512	512
		Immunofluorescence	128	128	128
		Gel Diffusion	+	+	+

Key * = serum titre

\+ = Positive reaction

Please amend the paragraph bridging lines 15-17 on page 14 in the following manner:

The results depicted in these Tables demonstrate on, the one hand, that ~~CastV-2~~ CAstV-2 strains form a homogeneous group of immunologically related viruses and, on the other hand, that that ~~CastV-2~~ CAstV-2 is immunologically distinct from the other avian astroviruses.

Please amend the paragraph bridging lines 15-17 on page 14 in the following manner:

Example 3 **Pathogenicity test in young chicks**

Twenty, day-old SPF chicks were inoculated with $10^{5.6}$ plaque forming units (PFU) of an isolate from strain ~~CastV-2~~ CAstV-2 strain a. The virus had been plaque-picked three times from a 10^{-4} dilution of the virus and had undergone a total of 6 CEL culture passages.

All inoculated chicks showed ~~diarrhoea~~ diarrhea and passed partly digested food in ~~faeces~~ feces. When 4 chicks were killed 5 days after infection they all showed some degree of distended small intestines.

The 17 control uninfected chicks showed no such signs and when 5 were killed at 5 days the intestines appeared normal.